^{13}C NMR studies of conformational dynamics in α-tocopherol esters in solution and solid state

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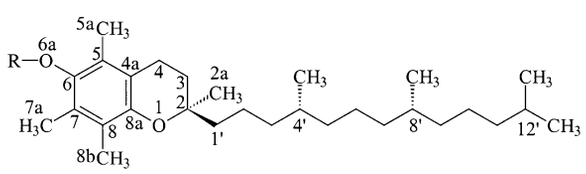
Molecular dynamics in esters of α-tocopherol were studied by means of dynamic ^{13}C NMR in solution and ^{13}C CP MAS NMR in solid phase. The coalescence of C2a, C3 and C1′ carbon signals was monitored and discussed in terms of hindered rotation around the C–O_{ester} bond. The dynamic parameters (k, $\Delta G^{\ddagger}$) were determined. The barrier was described by the interplay of steric and electronic effects of the ester residue. The molecular interaction of the 2,2,6,6-tetramethylpiperidinyloxyl radical as a spin probe with α-tocopheryl acetate at low temperature (263 K) was also investigated. The regiospecificity towards free radical approach was observed.

Introduction

It has been generally accepted that vitamin E, especially α-tocopherol (**1**), acts as a biological antioxidant in the lipid core of biomembranes where it is mostly located.[1,2] α-Tocopherol stabilises the lipid bilayers of membranes *via* van der Waals interaction with unsaturated fatty acid chains of phospholipids.[3,4] The effect has been studied using various techniques, such as ultraviolet and infrared absorption as well as electron spin resonance and nuclear magnetic resonance.[5,6] Srivastava *et al.* described a difference between the binding of α-tocopherol and tocopheryl acetate with model dipalmitoylphosphatidylcholine vesicles.[7] Following their observation, one can conclude that esterification of the phenolic group in α-tocopherol changes the molecular flexibility, which appears markedly different in interaction with fatty acids chains in phospholipid bilayers. Urano and Matsuo reported that tocopherol derivatives bearing no hydroxy group form a complex with fatty acid more easily than α-tocopherol.[8] The importance of the hydroxy group of α-tocopherol for determining its interaction with phospholipids has been also emphasized by Lai *et al.*[9] They observed that hemisuccinate† of α-tocopherol (**4**) showed a remarkably lower effect on the phospholipid phase transition than free α-tocopherol.



1: R = H
2: R = acetyl
3: R = nicotinyl
4: R = hemisuccinyl

We have recently reported some results of NMR investigations concerning the molecular dynamics and conformational preferences in 2,2,5,7,8-pentamethylchroman-6-ol (α-model compound) and its esters. An increase in rigidity of the ester molecules in comparison with free chroman-6-ol was observed.[10]

The aim of our investigations is to provide more information about conformational and dynamic effects occurring in tocopherols by means of ^{13}C NMR spectroscopy in solution and solid state. In our opinion, the obtained results may enrich our knowledge about the mechanism of incorporation of α-tocopherol into biological membranes. The structural and dynamic properties of α-tocopherol influence mobility and molecular orientation in phospholipid bilayers. The ^{13}C NMR technique in solution and in solid state provides a good method for the study on molecular dynamics.

Results and discussion

Dynamic NMR investigation

^{13}C NMR spectra of compounds **2**–**4** measured for $CDCl_3$ solutions at ambient temperature showed that signals of carbons C-3, C-2a and C-1′ were markedly broadened or even gave two completely separated singlets (Table 1). Surprisingly, no similar effect in free α-tocopherol (**1**) was observed.

It seems that esterification of the 6-OH group causes an increase of rigidity in comparison with free α-tocopherol (**1**). A similar effect was observed for the esters of 2,2,5,7,8-pentamethylchroman-6-ol; hindered rotation around the C–O_{ester} bond resulted in separate signals in the ^{13}C NMR spectra for the two *gem*-dimethyl groups.[10]

Ekiel *et al.* observed approximately equal populations of two interconverting conformers of α-tocopherol (**1**) in the ^{1}H NMR spectrum recorded in pyridine-d_5 solution.[11] This result was supported by the analysis of the ^{2}H spectra of deuterated analog, [3,4-$^{2}H_2$]. The most likely conformers of the heterocyclic ring are the two half chairs; the two forms: a) 2-*endo*-3-*exo*, and b) 2-*exo*-3-*endo* (*endo* means upward position of the atom from the average molecular plane) gave the best results for the assignment of splittings of deuterium resonances.[11] In the case of tocopherol esters, as for the chromanol derivatives, a hindered rotation around the C–O_{ester} bond enables observation of conformational interconversion. The largest differences in chemical shifts are for carbons directly bound to C2 (C3, C2a, and C1′), however the exchange is reflected in the more distant carbons (up to C4′) of the phytyl chain.

† The IUPAC name for α-tocopheryl hemisuccinate is α-tocopheryl hydrogen succinate.

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Table 1 ^{13}C NMR data for the esters of α-tocopherol (**2**–**4**)

	Acetate (**2**)		Nicotinate (**3**)		Hemisuccinate (**4**)			
						MAS		
Carbon atom	263 K, acetone δ (ppm)	263 K, acetone $d\Delta\delta/dc_r$	258 K, $CDCl_3$ δ (ppm)	MAS δ (ppm)	263 K, $CDCl_3$ δ (ppm)	δ (ppm)	T_{CP}/ms	$T_{1\rho}$/ms
2	75.65	0.19	74.97	75.5	74.94	75.1/73.0	0.58/0.55	58/48
3	31.80/31.69	2.59/2.62	30.84/30.56	33.1	30.99/30.80	31.61		
4	21.05	2.67	20.46	20.3	20.54	19.52		
4a	118.07	0.40	117.40	119.8	117.28	117.8/117.3	0.67	85
5	122.98	0.36	123.13	122.6	122.89	124.3	0.99	65
6	141.60	0.25	139.80	140.8	140.37	141.9	1.17	61
7	125.88	0.32	124.86	126.3	124.88	126.8	0.98	59
8	127.42	0.34	126.49	126.3	126.62	128.5	0.88	58
8a	149.70	0.19	149.43	150.0	149.31	149,0	1.22	68
5a	12.08	2.74	11.85	11.4	11.72	11.1	0.32	162
7a	13.18	2.86	13.04	12.6	12.81	12.2	0.37	152
8b	12.31	3.20	12.22	12.6	11.96	12.2	0.37	152
2a	24.32/23.96	3.01/3.20	24.75/24.35		24.13/23.64	23.5/23.1		
1′	40.65/39.99	1.67/1.89	39.14/38.37	42.7	40.20/39.42	46.6/45.0	0.12	77
2′	21.74/21.68	—[a] /1.12	20.90	20.3	20.99	20.5		
3′	37.96	1.22	37.08	36.92	37.20	40.3		
4′	33.45/33.33	0.82/0.84	32.57/32.50	33.09	32.76/32.70	34.9		
5′	38.13	1.50	37.26	36.92	37.37	40.4		
6′	25.16	1.24	24.35		24.44	25.1		
7′	38.13	1.50	37.26		37.37	40.4		
8′	33.52	0.80	32.64		32.76	33.7		
9′	38.13	1.50	37.26		37.37	40.4		
10′	25.59	1.22	24.75		24.83	26.4		
11′	40.05	1.54	39.14		39.28	41.6		
12′	28.80	1.08	27.86		27.97	29.0		
12a′	23.02	2.67	22.69		22.79	23.1		
13′	23.12	2.67	22.56		22.67	21.2		
4a′	20.15	2.59	19.64	18.2	19.75	18.6		
8a′	20.08	2.64	19.50	18.2	19.65	18.6		
1″ (6a) CO	169.55	0.06	163.87	164.35	176.08	181.8	0.57	68
2″ (6b)	20.51	6.38		155.5	28.67	27.5		
3″ (6c)	—	—		151.1	28.96	28.5		
4″ (6d)	—	—			170.94	171.1/170.5	0.58/0.59	85/122

[a] Overlapped with other signals.

In order to establish kinetic parameters of the observed phenomena, the ^{13}C NMR spectra of compounds **2**–**4** in the temperature range 253–320 K near coalescence points were recorded. The spectra for **4** in the range 20–44 ppm at lowered temperature, as an illustration, are presented in Fig. 1.

In the spectrum of **2** recorded at 263 K the signals of C2a, C3, C1′, C2′, C3′ and C4′ appeared as doublets separated by 0.36, 0.116, 0.68, 0.064, 0.165 and 0.121 ppm, respectively. Therefore multiple estimations of the life-time are possible from one spectrum. The rate constants obtained at several temperatures were used for determination of free energy of activation. The temperature range can be extended, because the kinetic data are available from temperatures below and above six coalescences, and the estimation of $\Delta G^{\ddagger}$ is more precise. Most kinetic data collected in Table 2 were obtained from the lineshape analysis of the C1′, C2a and C3 signals, which exhibited the largest splittings. The average values of $\Delta G^{\ddagger}$ for the coalescence temperature of the above resonances have been taken as a measure of a barrier, ascribed to rotation around the C6–O_{ester} bond.

The range of $\Delta G^{\ddagger}$ varied from 62.6 kJ mol^{-1} for the nicotinate **3** to 59.4 kJ mol^{-1} for the hemisuccinate **4**. It should be noted that the barriers ($\Delta G^{\ddagger}$), reported earlier for the acetate and the nicotinate of 2,2,5,7,8-pentamethylchroman-6-ol, were lower (59.5 and 61.8 kJ mol^{-1}, respectively),[10] but followed the same sequence. Higher barriers for the esters of α-tocopherol are observed, if compared with those for α-model compound. The replacement of the methyl group by an alkyl chain should not exert any significant effect on the remote ester group. The frozen rotation of an ester substituent results in differentiation of α (carbonyl group downward) or β (carbonyl group upward) sides of the chroman moiety. Similarly, in a molecule of α-tocopherol ester the 2a-methyl group and carbons of the phytyl chain up to C4′ can also be distinguished. The comparison of molecular geometry and electron density in the respective esters of chromanol and tocopherol should shed light onto this problem.

Table 2 Kinetic parameters for heterocyclic ring dynamics in **2**–**4**. Coalescence parameters are given in italic

Ester	T/K	τ/s	t/s^{-1}	$\Delta G^{\ddagger}$/kJ mol^{-1}
Acetate (**2**)	293	0.011	90.9	
	296	*0.0091*	*109.9*	*61.0* (for C2a)
	300	0.0064	156.3	
	301	*0.0061*	*164.0*	*60.9* (for C1′)
Nicotinate (**3**)	292	0.065	15.5	
	300	0.015	66.6	
	304	*0.0091*	*109.9*	*62.6* (for C2a)
	305	0.008	125.0	
	309	*0.061*	*164.0*	*62.6* (for C1′)
	310	0.056	178.6	
Hemisuccinate (**4**)	263	0.09	11.1	
	272	*0.032*	*32.2*	*58.6* (for C3)
	273	0.037	27.0	
	283	0.016	62.5	
	288	*0.0098*	*102.0*	*58.0* (for C2a)
	294	*0.061*	*163.9*	*59.4* (for C1′)

Interaction with TMPN radical

Aminoxyl radicals have been used as probes in the studies of molecular interactions.[12–14] The 2,2,6,6-tetramethylpiperidinyloxyl radical (2,2,6,6-tetramethylpiperidine nitroxide, TMPN)

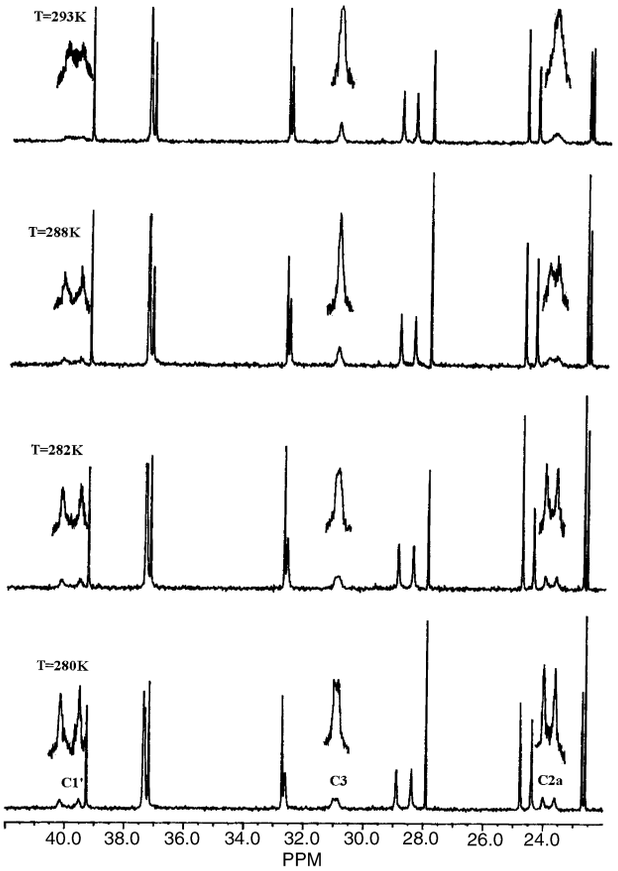

Fig. 1 ^{13}C NMR spectra of **4** recorded in the temperature range 280–293 K (22–42 ppm).

interacts with electron acceptors to form hydrogen bonded complexes and/or short living transient species (collision complexes) with hydrocarbons. The interaction (collisions) produces a positive spin density on ^{13}C nuclei and the observed chemical shift towards high frequency is mainly of contact nature. The shift depends on the number of protons (decreases in the order $CH_3 > CH_2 > CH > C$), and on the acidity of protons linked to carbon as well as on the steric hindrance.[13,14] It seemed worthwhile to check the potential utility of the aminoxyl spin probe in the elucidation of the properties of α-tocopherol derivatives. The spectra of **2** were recorded at 263 K, which was found to be a sufficiently low temperature to obtain well separated signals without exchange broadening. The chemical shifts δ_r induced by the radical were upfield and showed a linear dependence on the analytical concentration of the radical c_r. The slopes $d\Delta\delta/dc_r$, where $\Delta\delta = \delta_r - \delta_0$ were taken as a measure of TMPN interaction with tocopherol acetate. The smallest slopes (0.19–0.40) are for the quaternary carbons of the chroman part, as expected. The same small values of 0.19 were obtained for C2 and C8a, adjacent to O1. The approach of the radical is hindered due to the repulsion between electron-rich ring oxygen and negative charge on the oxygen of TMPN. There is no evident steric hindrance for the radical approach to the methyl or methylene groups of tocopheryl acetate (**2**), which should favour one over another. The methyl groups located on the phytyl chain are characterised by the slope 2.59–2.68, the same value of 2.67 was determined for both terminal methyls C12a′ and C13. Methyl groups of the chroman part, however, behave differently toward the radical because they differ in their electron-acceptor properties. The largest value of 6.38 is for the ester methyl group (acetate residue), and significant acceptor properties are exhibited by methyl groups C7a and C8b. The interaction with TMPN radical is in agreement with previous findings[15] that the chroman part showed regiospecificity towards oxidation, electrophilic substitution, and free radical alkylation. It is worth noting that in all doublets the carbon representing the low-frequency component is more sensitive to the interaction with the radical. In the case of C2a and C1′ these components can be assigned to the axial methyl carbon.

Solid state NMR

^{13}C NMR spectra for solid compounds **3** and **4** were recorded. The acetate **2** has low melting point and does not remain solid under high-speed rotation without additional cooling. The spectrum of hemisuccinate **4** showed sharp signals, which suggested well-ordered molecular structure. The splitting of signals into 1 : 1 doublets, best seen for C2, C4a and C5′ proved a polymorphism; two molecules are present in the asymmetric crystal unit. According to our knowledge and the Cambridge Structural Database, there is no X-ray structure of α-tocopherol esters. In order to get insight into molecular structure of polymorphs, variable time cross-polarisation experiments were performed. Selected results of the variable-contact cross-polarisation experiments are illustrated in Fig. 2.

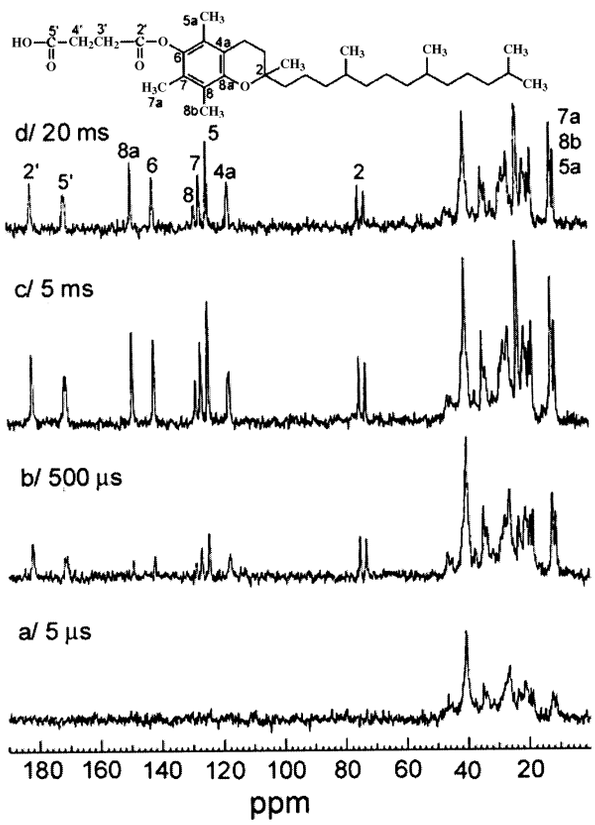

Fig. 2 ^{13}C CP MAS NMR spectra of α-tocopheryl hemisuccinate (**4**) recorded with various contact times (5 μs–20 ms).

For organic samples, the signal intensity in the CP spectra is a function of the contact time t: $I(t) = A[1 - T_{CP}/T_{1\rho}^{H}]^{-1}$ $[\exp(-t/T_{1\rho}^{H}) - \exp(-t/T_{CP})]$, where A is the intensity amplitude, T_{CP} is the time constant, which characterises the progress of cross polarisation and $T_{1\rho}^{H}$ (spin lattice relaxation time in the rotating frame) characterises the decay of signals.[16] The fitting with the above equation gave T_{CP} and $T_{1\rho}^{H}$ values, included in Table 1. The resonances of methylene carbons can be seen in the spectra recorded with a very short contact time (50 μs). The signals of aromatic carbons of the chroman part appear when contact time is longer that 0.5 ms. Maximum intensity is achieved with contact time of *ca.* 10 ms, which is longer than the 1–4 ms usually applied for organic crystals. Such a long contact time is mainly due to the presence of nine quaternary carbons and eight methyl groups, which probably undergo intramolecular rotation.

The T_{CP} values for quaternary carbons are 0.55–1.22 ms. A cross-polarisation time constant of 0.12 ms was obtained for C1′ (CH_2), shorter T_{CP} can be expected for CH_3 carbons since T_{CP} values decrease with the number of hydrogen atoms directly bound to the observed carbon atom. However, the values for methyl carbons are 0.32–0.37 ms, which confirm that methyl groups undergo intramolecular rotation (T_{CP} increases with increasing mobility). The $T_{1\rho}^{H}$ values are 48 to 85 ms for quaternary carbons and significantly longer 152–162 ms for rotating methyls. There is no remarkable difference in cross polarisation behaviour of the two forms present in the crystal. The values of T_{CP} and $T_{1\rho}^{H}$ obtained by fitting the intensity of two well resolved C2 resonances are similar.

In the spectrum of solid nicotinate **3** only the carbons from chroman part give narrow resonances, whereas broad signals in the range 32–42 ppm indicate that the hydrocarbon tail is disordered.

It is interesting to compare chemical shifts for solid compounds and for solution; significant differences $\Delta = \delta_{solution} - \delta_{solid\ state}$ indicate conformationally flexible fragments of the molecule. The largest Δ values are for 1′: −3.6/−4.3 ppm for the nicotinate **3** band −6.4/−6.9 or −4.8/−5.6 for the hemisuccinate **4**. The frozen conformation of the alkyl chain resulted in deshielding of carbons 3′ to 11′ of −3.1 to −1.0 ppm, a similar effect was observed for glycosyl derivatives of α-tocopherol.[17] A significant high frequency shift Δ = −5.7 ppm of C6a=O can be explained by the formation of hydrogen bonds, probably with the parent molecule of the hemisuccinate, in the solid phase.

The obtained results proved that the α-tocopherol esters are fairly flexible mostly in two fragments: at C2, where the phytyl chain is linked to chroman nucleus, and at C-6, where hindered rotation of ester group takes place. These dynamic phenomena are crucial for fitting tocopherols into the phospholipid membrane.

The chroman part, which is located in the outer sphere of the membrane, exhibited steric differentiation in the interaction with stable radicals.

Experimental

The esters of (+)-*d*-α-tocopherol: acetate **2**, nicotinate **3** and hemisuccinate **4** were purchased from Aldrich. The 2,2,6,6-tetramethylpiperidinyloxyl radical (TMPN) was prepared from 2,2,6,6-tetramethylpiperidine and purified by sublimation. ^{13}C NMR spectra were recorded using the Bruker ACF spectrometer (200 MHz) equipped with a variable temperature probe, for $CDCl_3$ solutions; chemical shifts (δ) are relative to TMS. The temperature stability was ±1°. In the experiments with TMPN the concentration of α-tocopheryl acetate in acetone was kept constant at 0.5 mol dm^{-3} and that of the radical varied from 0.05 to 0.14 mol dm^{-3}. The carbonyl signal of acetone was used as an internal reference of chemical shifts since it was not dependent on the radical concentration.[18] The line shape analysis was carried out using a computer program with an iteration procedure for a classical two-site exchange.[19] Cross polarisation magic angle spinning (CP MAS) solid state ^{13}C NMR spectra were recorded on a Bruker MSL 300 instrument at 75.5 MHz. The samples were spun at 10 kHz, a contact time of 4 ms, a repetition time of 6 s, and a spectral width of 20 kHz for accumulation of 700–900 scans were used. Chemical shifts were calibrated indirectly through the glycine C=O signal recorded at 176.0 ppm relative to TMS.

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